

CLAIMS

1. A method for regulating expression of a *tet* operator-linked gene in a cell of a subject, comprising:

5 introducing into the cell a nucleic acid molecule encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising a first polypeptide which binds to a *tet* operator sequence, operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells; and
10 modulating the concentration of a tetracycline, or analogue thereof, in the subject.

2. The method of claim 1, wherein the first polypeptide of the fusion protein binds to *tet* operator sequences in the absence but not the presence of tetracycline or a tetracycline analogue.

15 3. The method of claim 2, wherein the first polypeptide is a Tet repressor.

4. The method of claim 3, wherein the first polypeptide comprises an amino acid sequence shown in SEQ ID NO: 17.

20 5. The method of claim 1, wherein the first polypeptide of the fusion protein binds to *tet* operator sequences in the presence but not the absence of tetracycline or a tetracycline analogue.

25 6. The method of claim 5, wherein the first polypeptide is a mutated Tet repressor.

7. The method of claim 6, wherein the mutated Tet repressor has at least one amino acid substitution compared to a wild-type Tet repressor.

30 8. The method of claim 7, wherein the mutated Tet repressor has an amino acid substitution at at least one amino acid position corresponding to an amino acid position selected from the group consisting of position 71, position 95, position 101 and position 102 of a wild-type Tn10-derived Tet repressor amino acid sequence.

35 9. The method of claim 8, wherein the mutated Tet repressor comprises an amino acid sequence shown in SEQ ID NO: 19.

10. The method of claim 1, wherein the second polypeptide comprises a transcription silencer domain of a protein selected from the group consisting of v-erbA, the Drosophila Krueppel protein, the retinoic acid receptor alpha, the thyroid hormone receptor alpha, the

yeast Ssn6/Tup1 protein complex, the *Drosophila* protein even-skipped, SIR1, NeP1, the *Drosophila* dorsal protein, TSF3, SFI, the *Drosophila* hunchback protein, the *Drosophila* knirps protein, WT1, Oct-2.1, the *Drosophila* engrailed protein, E4BP4 and ZF5.

- 5 11. The method of claim 1, wherein the nucleic acid molecule encoding the fusion protein is integrated randomly in a chromosome of the cell.
12. The method of claim 1, wherein the nucleic acid molecule encoding the fusion protein is integrated at a predetermined location within a chromosome of the cell.
- 10 13. The method of claim 1, wherein the nucleic acid molecule encoding the fusion protein is introduced into the cell *ex vivo*, the method further comprising administering the cell to the subject.
- 15 14. The method of claim 1, wherein the *tet* operator-linked gene is an endogenous gene of the cell which has been operatively linked to at least one *tet* operator sequence.
15. The method of claim 1, wherein the *tet* operator-linked gene is an exogenous gene which has been introduced into the cells.
- 20 16. The method of claim 1, wherein the tetracycline analogue is anhydrotetracycline, doxycycline or cyanotetracycline.
- 25 17. A method for regulating expression of a gene in a cell of a subject, comprising:
obtaining the cell from the subject;
introducing into the cell a first nucleic acid molecule which operatively links a gene to at least one *tet* operator sequence;
introducing into the cell a second nucleic acid molecule encoding a fusion protein which inhibits transcription, the fusion protein comprising a first polypeptide which binds to a *tet* operator sequence, operatively linked to a second polypeptide which inhibits
30 transcription in eukaryotic cells, to form a modified cell;
administering the modified cell to the subject; and
modulating the concentration of a tetracycline, or analogue thereof, in the subject.
- 35 18. The method of claim 17, wherein the first polypeptide of the fusion protein binds to *tet* operator sequences in the absence but not the presence of tetracycline or a tetracycline analogue.
19. The method of claim 18, wherein the first polypeptide is a Tet repressor.

21. The method of claim 19, wherein the first polypeptide comprises an amino acid sequence shown in SEQ ID NO: 17.

22. The method of claim 20, wherein the first polypeptide of the fusion protein binds to *tet* operator sequences in the presence but not the absence of tetracycline or a tetracycline analogue.

23. The method of claim 17, wherein the first polypeptide is a mutated Tet repressor.

24. The method of claim 23, wherein the mutated Tet repressor has at least one amino acid substitution compared to a wild-type Tet repressor.

25. The method of claim 24, wherein the mutated Tet repressor has an amino acid substitution at at least one amino acid position corresponding to an amino acid position selected from the group consisting of position 71, position 95, position 101 and position 102 of a wild-type Tn10-derived Tet repressor amino acid sequence.

26. The method of claim 25, wherein the mutated Tet repressor comprises an amino acid sequence shown in SEQ ID NO: 19.

27. The method of claim 17, wherein the second polypeptide comprises a transcription silencer domain of a protein selected from the group consisting of v-erbA, the Drosophila Krueppel protein, the retinoic acid receptor alpha, the thyroid hormone receptor alpha, the yeast Ssn6/Tup1 protein complex, the Drosophila protein even-skipped, SIR1, NeP1, the Drosophila dorsal protein, TSF3, SFI, the Drosophila hunchback protein, the Drosophila knirps protein, WT1, Oct-2.1, the Drosophila engrailed protein, E4BP4 and ZF5.

28. The method of claim 17, wherein the nucleic acid molecule encoding the fusion protein is integrated at a predetermined location within a chromosome of the cell.

29. The method of claim 17, wherein the first nucleic acid molecule operatively links an endogenous gene of the cell to at least one *tet* operator sequence.

30. The method of claim 17, wherein the first nucleic acid molecule comprises a gene operatively linked to at least one *tet* operator sequence.

31. The method of claim 17, wherein the tetracycline analogue is anhydrotetracycline, doxycycline or cyanotetracycline.